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Original Article

Detection of Carbapenemases Emerging *in Acinetobacter baumannii* Clinical Isolates by Modified Hodge Test

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Introduction: *Acinetobacter baumannii*, a known causative agent of nosocomial infections, is one of the highly antibiotic-resistant gram-negative bacilli. Carbapenem-resistant *Acinetobacter* isolates are increasingly reported worldwide. Carbapenems such as imipenem and meropenem are efficient antimicrobial agents commonly used for the treatment of infections caused by multi-resistant *A. baumannii* strains. Some reports indicate treatment failure due to antibiotic resistant *A. baumannii* strains. The aim of this study was to determine antibiotic resistance pattern and prevalence carbapenemase production in *A. baumannii* isolates. **Method:** A total of 100 *A. baumannii* isolates were identified from clinical specimens by standard chemical tests. The samples were collected from the patients hospitalized in two teaching hospitals of Ahvaz, southwest of Iran. The susceptibility of isolates to different antibiotics was determined by the disk diffusion method based on Clinical Laboratory Standards Institute (CLSI) direction. The Modified Hodge Test (MHT) was performed for detection of carbapenemase - producing *A. baumannii* isolates. **Results:** The isolates showed the highest resistance to ciprofloxacin (98%). The resistance rate to cefotaxime, ceftazidime, and piperacillin was 97%, gentamicin, amikacin, and meropenem 96%, imipenem 95%, cefepime 93%, and tetracycline 60%. Most of the isolates (99%) were sensitive to colistin. Among the 100 *A. baumannii* isolates, 53 (53%) were positive for carbapenemase production by MHT. **Conclusion:** This study emphasizes dissemination of carbapenem resistant *A. baumannii* strains. Our study also showed that the MHT has an excellent sensitivity for detecting carbapenemase producing *A. baumannii* isolates. *J Med Microbiol Infec Dis, 2014, 2 (4): 163-166.*

Keywords: *Acinetobacter baumannii*, Antibiotic Resistance, Carbapenemase, Modified Hodge Test.

INTRODUCTION

Acinetobacter baumannii is recognized as an increasingly important opportunistic non-fermentative Gram-negative pathogen that is frequently associated with nosocomial outbreaks worldwide [1]. *A. baumannii* is often related to pneumonia, urinary tract, bloodstream, wound, and nosocomial meningitis infections [2].

This bacterium has the propensity to acquire resistance rapidly to various classes of antimicrobial agents and so shows high rates of resistance to multiple antimicrobial agents [1, 2]. There is ongoing controversy as to whether carbapenem resistance results in an increased risk of mortality in patients infected with *A. baumannii*. The emergence of carbapenem-resistant *A. baumannii* is a significant public health concern as there are limited alternatives for successful treatment [2, 3]. Resistance to carbapenem is mostly due to the production of enzymes-carbapenemases that hydrolysed carbapenems and other β-lactams. Carbapenemase enzymes fall into Ambler classification -A, B and D [4]. Unfortunately, resistance to carbapenems in *A. baumannii* is hard to detect by the routine disc diffusion method used by many microbiology laboratories.

Modified Hodge Test (MHT) is a phenotypic method for detection of carbapenemases. This approach is relatively easy and straightforward and is widely used as a screening test for detection of carbapenemases in Gram-negative bacteria [5].

In this study, we assessed the frequency of drug resistance in *A. baumannii* isolates originating from different clinical specimens in two teaching hospitals in Ahvaz, southwest of Iran. Also, carbapenemase production was assessed in carbapenems- resistant isolates by MHT.

MATERIAL AND METHODS

A total of 120 isolates of *Acinetobacter* were collected from patients hospitalized in Golestan and Emam Khomeini hospitals, Ahvaz city, Iran from December 2014 to October 2015. *A. baumannii* was the most prevalent species (83.3%) isolates. The reminder of them (16.7%) identified as *Acinetobacter* spp.

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The isolates were obtained from different specimens, including tracheal aspirate, wound, blood, urine, abscess, and CSF. All isolates were identified by biochemical tests including Gram stain, oxidase test, fermentative and oxidative properties, growth at 42˚C, and citrate and malonate tests [6].

The susceptibility test of bacteria was performed by disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines. *Pseudomonas aeruginosa* ATCC27853 was used as the quality control strain. The isolates with resistance to imipenem were examined for the production of carbapenemases by the MHT. This test was performed according to CLSI guidelines [7].

Antimicrobial susceptibility test to imipenem (IPM: 10 µg), meropenem (MEM:10 µg), ceftazidime (CAZ: 30 µg), cefotaxime (CTX: 30 µg), cefepime (FEP: 30 µg), ciprofloxacin (CIP: 5 µg), piperacillin (PIP, 100 µg), amikacin (AK: 30 µg), gentamicin (GEN: 30 µg), tetracycline (TE: 30 µg) and colistin (CO, 10 µg) (MAST, UK) was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) (Merck, Germany) based on CLSI Guidelines 2014 [7].

The carbapenem-resistant isolates were evaluated for carbapenemase production by MHT as CLSI recommended. *Escherichia coli* ATCC25922 at a turbidity equivalent to 0.5 McFarland standard was inoculated onto the Mueller-Hinton agar (MHA) (Merck, Germany) plate. After brief drying for five min, ertapenem (10 µg) disc was placed at the center of the plate. The test strain was heavily streaked from the edge of the ertapenem disc to the periphery of the plate. Four isolates were inoculated in one plate at 90˚ to each other, and the plates were incubated at 35˚C for 18-24 h. *Klebsiella pneumoniae* ATCCBAA -1705 was used in this study as a quality control.

The presence of a clover leaf type of zone of inhibition near the test organism was interpreted as positive for carbapenem-hydrolyzing enzymes, as per the CLSI guidelines. MHT negative test did not show clover leaf pattern near to the test organism [7- 9].

RESULTS

In this study we examined the clinical specimens which were collected from 120 patients, out of 120 clinical isolates of the genus *Acinetobacter*, 100 were *A. baumannii* including 58 (58%) from men and 42 (42%) from women. The majority of *A. baumannii* isolates were obtained from tracheal aspirate (46%) followed by blood (17%), lung discharge (13%), wound (9%), CSF (7%), urinary tract (5%) and sputum (3%) specimen. The isolates showed the highest resistance to ciprofloxacin (98%). The resistance rates to other antibiotics were as follows: cefotaxime, ceftazidime and piperacillin (97%); gentamicin, amikacin and meropenem (96%); imipenem (95%); cefepime (93%); and tetracycline (60%). Most of the isolates (99%) were sensitive to colistin (Table 1).

Among imipenem resistant *A. baumannii* isolates, 53 (53%) showed carbapenemase production by MHT (Table 2 and Figure 1).

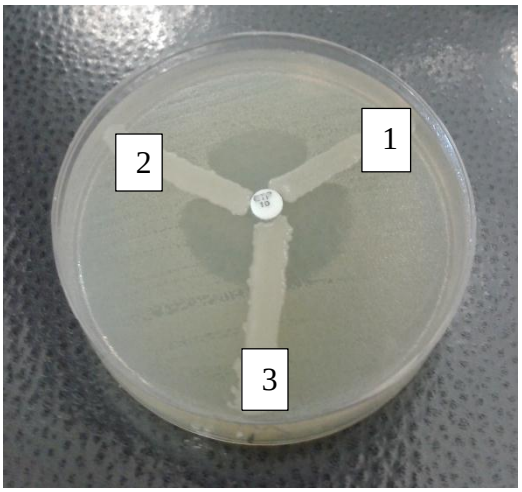

Fig. 1. MHT with ertapenem disks.

1. Quality control (MHT Positive *K. pneumoniae* ATCCBAA -1705); 2. Positive result, A positive MHT indicates that this isolate is producing a carbapenemase; 3. MHT negative result for carbapenemase detection by MHT.

Table 1. Result for antimicrobial susceptibility test against *A. baumannii* isolates.

Antibiotic	Resistant NO. (%)	Intermediate NO. (%)	Sensitive NO. (%)
Imipenem	**95 (95)**	**1 (1)**	**4 (4)**
Meropenem	**96 (96)**	**0 (0)**	**4 (4)**
Ceftazidime	**97 (97)**	**0 (0)**	**3 (3)**
Cefotaxime	**97 (97)**	**3 (3)**	**0 (0)**
Cefepime	**93 (93)**	**1 (1)**	**6 (6)**
Ciprofloxacin	**98 (98)**	**0 (0)**	**2 (2)**
Amikacin	**96 (96)**	**0 (0)**	**4 (4)**
Piperacillin	**97 (97)**	**0 (0)**	**3 (3)**
Gentamicin	**96 (96)**	**0 (0)**	**4 (4)**
Tetracycline	**60 (60)**	**12 (12)**	**28 (28)**
Colistin	**1 (1)**	**0 (0)**	**99 (99)**

Table 2. Sensitivity and resistance of clinical isolates of *A. baumannii* to ertapenem

Antibiotic	Resistant NO. (%)	Intermediate NO. (%)	Sensitive NO. (%)
Ertapenem	**53 (53)**	**22 (22)**	**25 (25)**

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DISCUSSION

A. baumannii is one of the most common known agents in nosocomial infections in hospitals. Management of *A. baumannii* infections has become a great challenge for physicians due to its survival for an extended period of time on various surfaces and development of resistance extends to multiple drug agents [10]. *Acinetobacter* spp. can survive in hospitals milieu for long periods and this bacteria could cause serious infections, especially in immune suppressed hosts, patients in the intensive care unit and those with critical disorders [11, 12]. In our study, most of (46%) *A. baumannii* isolates were obtained from tracheal secretions followed by blood (17%), discharge (13%). Other isolates of *A. baumannii* were derived from the wound infection (9%), CSF (7%), urinary tract (5%) and sputum (3%). In a similar study, by Begum *et al.* (2013) most of the *A. baumannii* isolates were obtained from endotracheal tube specimens followed by tracheal secretions and pus [13]. Shanthi *et al.* (2009) have described that the highest prevalence of *A. baumannii* was found from the respiratory tract (41.8%) followed by urinary tract (25.5%), wound (20%) and blood (12.7%) [14].

Acinetobacter infections require complex treatment because isolates of this bacteria resistant to most of the available antibiotics. Carbapenems such as imipenem and meropenem have a broad spectrum of activity against some of the bacteria, and they resist to hydrolysis by many of β. lactamases [15]. The reports show the mechanism resistance to carbapenem mostly is due to the production of enzymes carbapenemases that hydrolysed carbapenems and other β-lactams [16]. However, development of resistant to these drug agents in *Acinetobacter* have been rising during recent years, and the isolates of these bacteria are often multiple drug resistance (MDR) [17].

Carbapenemase production is determined by the MHT which originally has been recommended by the Center for Disease Control and Prevention (CDC), and it has revealed appropriate efficiency for carbapenemase detection [9, 15].

In the present study, most of the *A. baumannii* isolates were resistant to cephalosporins, carbapenems, aminoglycosides, ciprofloxacin and tetracycline, and susceptible to colistin.

Vahdani and colleagues (2011) reported a high frequency of cephalosporin (more than 90%) and fluoroquinolone (80%) resistance in *A. baumannii* isolates in Tehran [18]. Also, Asadollahi and colleagues (2012) showed that the resistance rate to β-lactam antibiotics, ciprofloxacin, and tetracycline was more than 50% and to imipenem 47.8% in Tehran. Unlike our results, antibiotic resistant was reported on the lower level in this study [19].

Fallah *et al.* (2013) recorded that resistance rate of *A. baumannii* isolates were 95.4%, 91.7%, 91.7%, 80.6%, and 92.6% to ceftazidime, meropenem, imipenem, amikacin, and ciprofloxacin in Tehran [20]. Also Shoja *et al.* (2013) reported the resistant rate of *A. baumannii* isolates to ciprofloxacin, piperacillin 96.6%, to imipenem, meropenem, ceftazidime and cefepime 96.1%, amikacin 88.8%, tetracycline 85% and gentamicin 83% [21].

Our result in the present study agrees with other studies [18, 20, 21] which have all revealed that resistance to most antimicrobial drugs, especially carbapenems have increased during recent years and so it can lead to failure in the clinical treatment of *A. baumannii* infections.

Carbapenemase production detected in this survey by MHT was 53%. Hence, this test could determine about half of positive carbapenemase isolates.

This study shows that MHT could be used in clinical laboratories for monitoring emergence carbapenemase in *A. baumannii*, especially MDR isolates.

In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming rampant, complicating treatment and increasing both human morbidity and financial costs. This necessitates the need for detecting the resistant bacteria so that unnecessary use of broad-spectrum antimicrobials can be avoided [5].

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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